Adv Ther
https://doi.org/10.1007/s12325-020-01571-z

REVIEW

Identifying Novel Biomarkers Ready for Evaluation in Low-Prevalence Populations for the Early Detection of Upper Gastrointestinal Cancers: A Systematic Review

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Received: October 5, 2020 / Accepted: November 11, 2020

ABSTRACT

Introduction: Detecting upper gastrointestinal (GI) cancers in primary care is challenging, as cancer symptoms are common, often non-specific, and most patients presenting with these symptoms will not have cancer. Substantial investment has been made to develop biomarkers for cancer detection, but few have reached routine clinical practice. We aimed to identify novel biomarkers for upper GI cancers which have been sufficiently validated to be ready for evaluation in low-prevalence populations.

Methods: We systematically searched MEDLINE, Embase, Emcare, and Web of Science for studies published in English from January 2000 to October 2019 (PROSPERO registration CRD42020165005). Reference lists of included studies were assessed. Studies had to report on second measures of diagnostic performance (beyond discovery phase) for biomarkers (single or in panels) used to detect pancreatic, oesophageal, gastric, and biliary tract cancers. We included all designs and excluded studies with less than 50 cases/controls. Data were extracted on types of biomarkers, populations and outcomes. Heterogeneity prevented pooling of outcomes.

Results: We identified 149 eligible studies, involving 22,264 cancer cases and 49,474 controls. A total of 431 biomarkers were identified (183 microRNAs and other RNAs, 79 autoantibodies and other immunological markers, 119 other proteins, 36 metabolic markers, 6 circulating tumour DNA and 8 other). Over half (n = 231) were reported in pancreatic cancer studies. Only 35 biomarkers had been investigated in at least two studies, with reported outcomes for that individual marker for the same tumour type. Apolipoproteins (apoAII-AT and apoAII-ATQ), and pepsinogens (PGI and

Electronic Supplementary Material The online version of this article (https://doi.org/10.1007/s12325-020-01571-z) contains supplementary material, which is available to authorized users.

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Published online: 11 December 2020

PGII) were the most promising biomarkers for pancreatic and gastric cancer, respectively.
Conclusion: Most novel biomarkers for the early detection of upper GI cancers are still at an early stage of matureness. Further evidence is needed on biomarker performance in low-prevalence populations, in addition to implementation and health economic studies, before extensive adoption into clinical practice can be recommended.

Keywords: Biomarkers; Clinical practice; Early detection; Primary care; Upper gastrointestinal cancers

Key Summary Points

We aimed to identify novel biomarkers which had been validated and showed sufficient promise to warrant further evaluation in low-prevalence populations.

We identified 431 unique biomarkers; only 35 of which had been investigated in at least two studies, with outcomes for that individual marker for the same tumour type - four of these were identified as the most promising for future studies.

This review highlights the need for more biomarker studies that consider primary care/community settings as their intended populations.

Findings also indicate we still need better reporting to facilitate knowledge translation; we also need more consistency in the use of biomarkers.

Research collaborations are vital to reduce duplicate efforts and ensure appropriate samples sizes when studying low-prevalence populations.

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13214843.

INTRODUCTION

Gastrointestinal (GI) cancers represented more than 25% (4.8 million) of cancer cases and over a third (3.4 million) of cancer-related deaths worldwide in 2018 [1]. Upper GI cancers contribute an important proportion of these, with over 2.1 million new cases of cancers of the stomach, oesophagus, pancreas and biliary tract diagnosed worldwide in 2018 [1, 2]. Prognosis is often poor as upper GI cancers are generally not detected until the disease is advanced and less amenable to curative treatment [1].

Primary care plays a key role in the early detection of upper GI cancers, as more than 90% of patients present with symptoms [3–5], and screening tests for asymptomatic populations are not yet widely established. Early detection of upper GI cancers is challenging, as initial symptoms such as indigestion, abdominal discomfort or fatigue are common, often intermittent, and most patients presenting with them will not have cancer [6, 7].

There is growing demand to improve early cancer detection through better diagnostic and triage approaches, particularly for use in primary care or other community settings where cancer prevalence is low [5]. New diagnostic approaches, applied either among asymptomatic at-risk populations or to triage patients presenting with cancer symptoms, could be transformational. Electronic health records and large population-based surveys have been used to develop cancer risk prediction models to

identify those requiring investigation for cancer [8]; diagnostic pathways have also been implemented in different countries in an effort to improve timely cancer diagnosis [5]. Innovative strategies applying artificial intelligence techniques to imaging and other medical data are also promising [5, 9]. For cancers with non-specific symptom signatures, like most upper GI cancers, we also need better biomarkers to support diagnostic assessment [10]. Biomarkers such as carcinoembryonic antigen (CEA) and CA19-9 are used in clinical practice predominantly for surveillance following treatment of upper GI cancers [9, 11]. Substantial investment has been made into developing new biomarkers for early cancer detection; most such biomarker research has been conducted in laboratory and specialist clinical settings [12, 13], where cancer prevalence is higher compared to community settings [14, 15].

The distinction between care settings is important, as the diagnostic performance characteristics of a test are strongly determined by the prevalence and severity of the target disease and of other diseases within the study population [14]. In populations in which the prevalence of the target disease is low (e.g. primary care), positive predictive values are lower than in high-prevalence populations seen in specialist cancer centres. Tests evaluated in high-prevalence populations tend to have lower sensitivity and higher specificity when used in low-prevalence populations [15, 16]. This is known as the spectrum effect or spectrum bias [14, 15] and has crucial implications for translating results from one care setting to another. To gain an accurate understanding of how a test will perform within a low incidence setting, it must ultimately be evaluated within that setting.

In recognition of this, the CanTest Framework has been developed, proposing a 5-phase translational pathway for diagnostic tests, from new test development to health system implementation in low-prevalence populations [15]. The framework highlights the importance of evaluating not only clinical performance but also the feasibility and acceptability of implementation, patient safety and quality of care, and cost-effectiveness in the chosen clinical setting. Understanding and addressing these issues is vital, as test performance alone, even if evaluated in the target populations, does not guarantee clinical utility nor improved patient outcomes [12].

This review set out to systematically identify novel biomarkers for the early detection of upper GI cancers which have been validated and show sufficient promise to warrant further evaluation in low-prevalence populations.

METHODS

Search Strategy and Inclusion/Exclusion Criteria

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [17], and the protocol was registered in PROSPERO (CRD42020165005). We searched MEDLINE, Embase, Emcare and Web of Science from 1 January 2000 to 31 October 2019 for primary studies published in English. The search strategy (Online supplementary file 1) was developed with the assistance of a medical librarian and refined until it identified all relevant core publications known by the senior authors. Reference lists of included studies were also screened. Articles that were not available online were ordered via the British Library.

Studies were included if they reported on at least one measure of diagnostic performance: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive, false negative or area under the curve (AUC) for biomarkers used to detect oesophageal, gastric, pancreatic or biliary tract cancers. We included adult populations (mean/median age $\geq$ 18); we accepted individuals aged $<$ 18 if these were outliers in large samples. The search strategy also included terms for lower GI (colorectal and anal) cancers for the purposes of a parallel review of novel biomarkers for the early detection of lower GI cancers, to be reported separately. Non-specified GI cancers, neuroendocrine cancers and studies only reporting on familial populations at risk of hereditary cancers were excluded.

Novel biomarkers were considered both individually and as part of a combination/panel test. Studies reporting only the performance of a single, established biomarker (i.e. CEA and CA19-9 for pancreatic cancer) were not eligible for inclusion [9]. We included studies reporting on performance for established biomarkers if these were in combination with additional novel biomarkers.

We aimed to identify studies situated within Phase 2 (measures of diagnostic accuracy in high-prevalence settings) and Phase 3 of the CanTest framework (measures of diagnostic accuracy or clinical utility, acceptability and feasibility in intended low-prevalence settings) (Fig. 1) [15]. We included studies if they reported more than preliminary measures of performance calculated in a discovery phase; this required additional measures of diagnostic performance in an independent cohort. If no references to previous studies evaluating performance were available and the study provided only one set of measures, the study was excluded. Panels with previously investigated biomarkers were included even if the biomarkers had not been investigated as part of a panel. As larger sample sizes are required beyond the biomarker discovery phase [13, 18], studies had to include at least 50 cancer cases and at least one group of 50 non-cancer controls with similar clinical characteristics (healthy, or with non-malignant or pre-malignant conditions). Similar criterion has been adopted by previous reviews that informed our study [13, 19].

We only included biomarkers which are feasible to use in a community setting, i.e. blood (serum and plasma), urine, faecal, salivary or breath samples. Observational studies (cross-sectional or longitudinal, prospective or retrospective) and trials were eligible for inclusion.

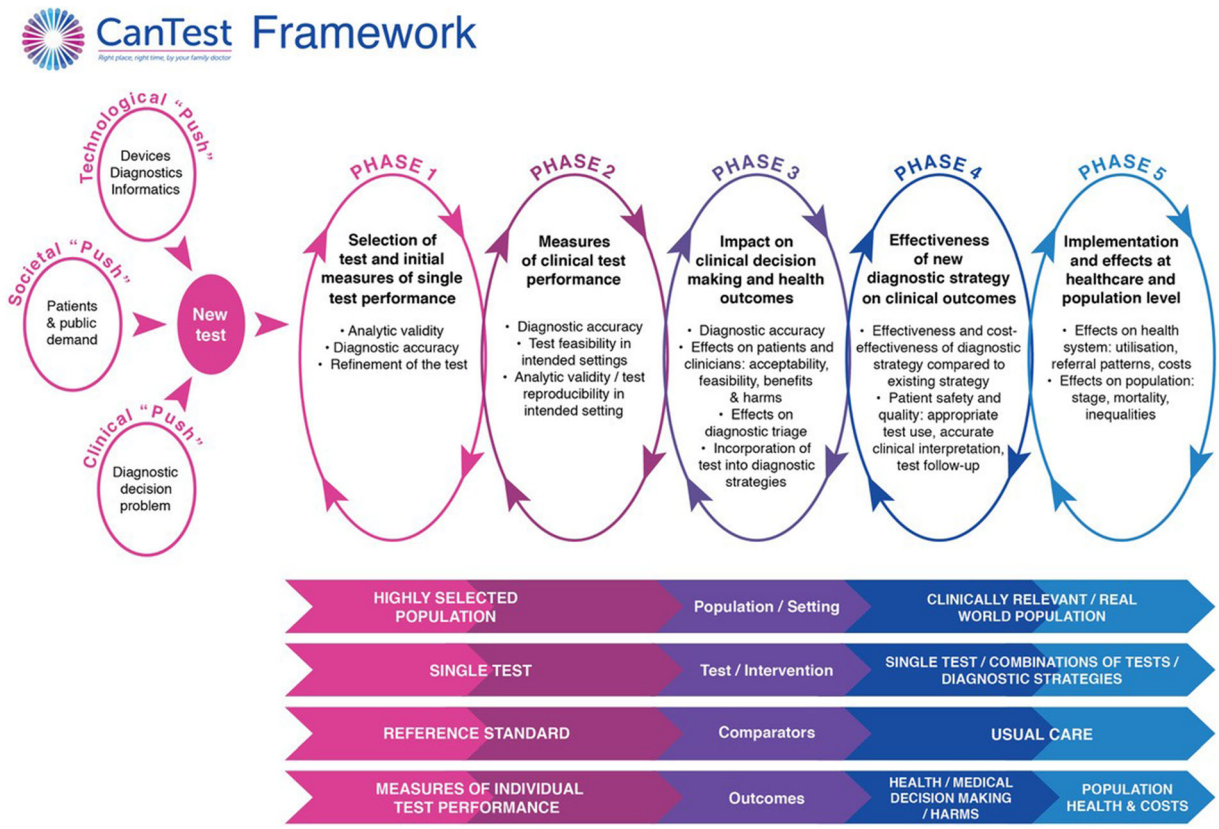


Fig. 1 The CanTest Framework Reproduced with permission from [15]

We included all recruitment settings, as we expected that very few studies would have been carried out in community settings.

We used the online tool Covidence [20] to facilitate title and abstract screening and study selection. Two reviewers (any two of NC, PED, CS, KMM, DB or RB) independently screened titles and abstracts. Then, two reviewers (any two of the above) independently evaluated full-text articles for inclusion. Titles and abstracts of reference lists of included studies were reviewed by one author (NC); full-text articles selected at this stage were independently assessed by two reviewers (any two of NC, PED, RB or DB). Disagreements were resolved by consensus; when this could not be reached a senior, third reviewer (FMW or JE) was consulted.

Data Extraction and Analysis

Data extraction was piloted to ensure consistency and was carried out by one of seven reviewers (NC, PED, RB, DB, JMG, JO and SS). We extracted information on: study characteristics (publication year, country of population of interest, recruitment setting, study aims and design); populations (numbers included, age, sex, tumour staging for cases and health status for controls); biomarkers (type of sample, biomarker name, biomarker category); and summary measures of diagnostic performance (sensitivity, specificity, PPV, NPV, false positives, false negatives and AUC, with 95% confidence intervals when available, for all comparisons). When studies reported on different phases of biomarker development, we only extracted data from the eligible phases (i.e. biomarkers and measures beyond the discovery phase). When studies had more than one eligible phase, we extracted data from all phases. Extracted data were collated and checked for consistency and inaccuracies (NC).

Biomarkers were categorised according to a modified version of Uttley et al.'s classification [19], which included: microRNAs and other RNAs, autoantibodies and other immunological markers, other proteins (that did not fit into other categories), metabolic markers, circulating tumour DNA, and other biomarkers. Controls were classified as: normal/healthy, having non-malignant, or pre-malignant conditions. Biomarkers and control populations were coded by one author (NC) and checked by other authors (PED, KMM and MM; and PED, FMW and JE, respectively). Controls described as being healthy were coded as such unless studies described underlying conditions. Patients with cancer were ineligible as controls. Full details of the classification of controls are available (online supplementary table S1). Microsoft Excel 2015 and SPSS v.23 (IBM) were used for data extraction and data analysis.

Quality Assessment and Risk of Bias

Risk of bias [21] was not assessed as described in the original protocol, following independent piloting. Appraisal was hindered by the use of diverse methods across studies and incomplete reporting, resulting in a large number of "unclear" assessments. Instead, a list of issues identified in the studies was prepared (Online supplementary file 2). As spectrum bias is a key issue when translating results from high- to low-prevalence populations, all included studies were classified as either single-gate or two-gate designs. In single-gate designs, cases and controls are recruited through a single route of entry and with the same inclusion criteria (e.g. all cases and controls presented with symptoms). In two-gate designs, participants are recruited through different routes and different inclusion criteria exist for cases and controls. In this situation, controls can be either normal/healthy or with an alternative diagnosis, which can produce symptoms and signs similar to patients with cancer [16]. One author (NC) classified all studies and another (PED) checked the classification. A full description of this classification and how it approaches some of the issues covered by the critical appraisal tool is available (Online supplementary file 3).

Data Synthesis

Included studies were heterogeneous and rarely evaluated the same biomarkers in the same way, often using different cut-off points, populations and/or biomarker combinations in panels. Therefore, we were unable to undertake meta-analysis. Instead, we used narrative synthesis to summarise data across studies [22]. First, we developed an overview of the available evidence, describing key characteristics of included studies, their populations and biomarkers, and outcome measures. Then, we looked for similarities that would allow for subgroup analyses, namely the same biomarker, for the same tumour type, with similar designs, outcome measures and populations.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

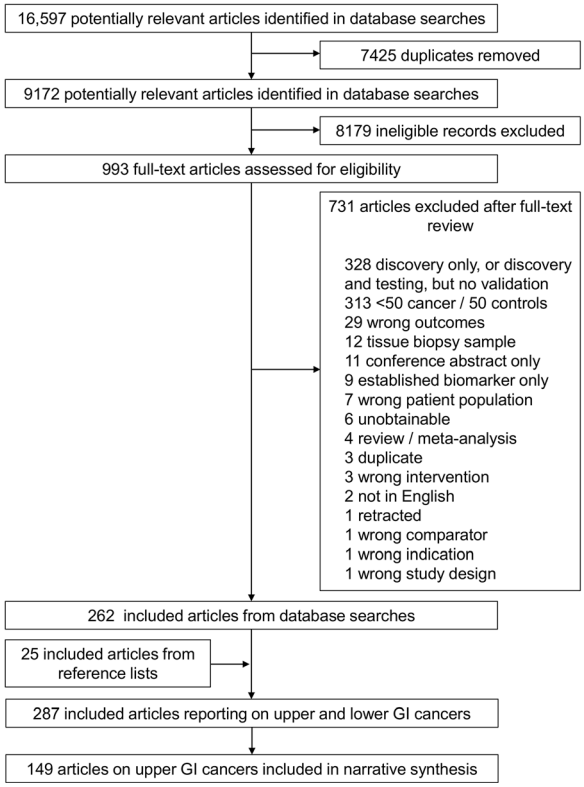


Fig. 2 Study selection

RESULTS

Database searches identified 16,597 records; 9172 were retained after removing duplicates. During title and abstract screening, 8179 ineligible records were excluded. The full texts of the remaining 993 records were assessed for eligibility; 731 were excluded (Fig. 2). A total of 262 studies from database searches met inclusion criteria; 25 additional studies were identified in reference lists. Of these, 149 included studies referred to upper GI cancers and were included in our narrative synthesis.

Characteristics of Included Studies

Key characteristics of included studies are described in Table 1 and 2. Most studies recruited participants from a single country (n = 142). China was the most common country (n = 77), followed by Japan and South Korea (n = 15 each), the USA (n = 12) and Germany (n = 9). The most common recruitment settings were hospital or other secondary care institutions (n = 125), biobanks, reference sets, databases or archived samples (n = 20), general population cohorts or cohorts from population screening programmes (n = 11) and cohorts from previous trials or observational studies (n = 9). Several studies recruited from more than one setting. Gastric cancer was the most commonly investigated tumour type (n = 69), followed by pancreatic (n = 54), oesophageal (n = 24) and biliary tract cancers (n = 3). Four studies investigated more than one type of upper GI cancer (Table 1).

Characteristics of Cases and Controls

Overall, the included studies reported on 22,264 cancer cases (10,589 gastric, 7964 pancreatic, 3258 oesophageal and 290 biliary tract cancers, and 163 oesophago-gastric cancers, not

Table 1 Characteristics of included studies: country, setting and population

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Gastric cancer only								
Cai et al. [23]	China	×	–	60	60	60	0	0
Chen et al. [24]	China	×	×	249	1203	0	1203	0
Chen et al. [25]	China	×	–	87	105	40	65	0
Chung et al. [26]	South Korea	×	–	147	94	U[b]	U[b]	24
Ding et al. [27]	China	×	–	110	110	110	0	0
Dong et al. [28]	China	×	–	90	57	57	0	0
Gantuya et al. [29]	Mongolia	×	–	50	752	0	752	0
Gwak et al. [30]	South Korea	U	U	96	187	0	187	0
He et al. [31]	China	×	–	149	235	124	111	0
Hoshino et al. [32]	Japan	–	×	248	74	74	0	0
Huang et al. [33]	China	×	–	197	125	37	88	0
Huang et al. [34]	China	×	×	62	59	59	0	0
Huang et al. [35]	China	×	–	60	60	60	0	0
Iwasaki et al. [36]	Japan	×	–	54	54	54	0	0
Ji et al. [37]	China	×	–	168	74	74	0	0
Juan Cai et al. [38]	China	×	–	106	358	160	198	0
Kaise et al. [39]	Japan	×	–	187	561	561	0	0
Kang et al. [40]	South Korea	×	–	380	626	228	291	107
Kikuchi et al. [41]	Japan	×	–	122	178	79	99	0
Kim et al. [42]	South Korea	×	–	120	120	U[b]	U[b]	0
Kurilovich et al. [43]	Russia	–	×	52	104	104	0	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Li et al. [44]	China	×	–	60	60	60	0	0
Li et al. [45]	China	×	–	79	112	81	0	31
Li et al. [46]	China	×	–	65	65	65	0	0
Li et al. [47]	South Korea	×	–	100	100	100	0	0
Li et al. [48]	China	×	–	234	428	270	0	158
Lim et al. [49]	South Korea	×	–	100	90	U[b]	U[b]	30
Lim et al. [50]	South Korea	×	–	100	100	U[b]	U[b]	30
Lin et al. [51]	China	U	U	51	78	60	18	0
Liu et al. [52]	China	×	–	142	105	105	0	0
Liu et al. [53]	China	×	–	119	148	99	49	0
Liu et al. [54]	China	×	–	50	50	50	0	0
Meistere et al. [55]	Taiwan, Latvia, Lithuania, Germany	×	×	829	929	929	0	0
Mroczko et al. [56]	Poland	×	–	73	61	61	0	0
Ning et al. [57]	China	×	–	169	75	75	0	0
Oue et al. [58]	Japan	×	–	123	96	76	20	0
Pan et al. [59]	China	×	–	81	130	77	53	0
Park et al. [60]	South Korea	×	–	81	103	32	63	8
Parvaee et al. [61]	Iran	–	×	50	50	50	0	0
Qin et al. [62]	China	×	×	407	407	407	0	0
Qiu et al. [63]	China	×	–	200	200	200	0	0
Song et al. [64]	China	–	×	68	68	0	68	0
Su et al. [65]	China	×		82	59	50	9	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Sun et al. [66]	China	×	×	332	332	332	0	0
Tsalikidis et al. [67]	Greece	×	–	99	78	78	0	0
Wang et al. [68]	Taiwan	U	U	170	116	116	0	0
Wang et al. [69]	China	×	–	72	54	54	0	0
Wang et al. [70]	China	×	×	186	186	186	0	0
Wang et al. [71]	China	×	–	60	120	60	60	0
Werner et al. [72]	Germany	–	×	146	97	97	0	0
Wu et al. [73]	China	×	–	90	90	90	0	0
Wu et al. [74]	China	×	–	99	132	100	30	2
Wu et al. [75]	China	×	–	201	318	157	161	0
Yanaoka et al. [76]	Japan	–	×	63	5146	5146	0	0
Yang et al. [77]	South Korea	–	×	290	290	290	0	0
Yang et al. [78]	China	×	–	109	106	0	106	0
Yoon et al. [79]	South Korea	×	×	500	200	200	0	0
Yun et al. [80]	China	×	–	194	376	185	191	0
Zayakin et al. [81]	Latvia, Germany	×	–	235	367	213	154	0
Zhang et al. [82]	China	×	–	114	298	187	111	0
Zhang et al. [83]	China	×	×	80	70	0	70	0
Zhang et al. [84]	China	×	–	80	80	0	80	0
Zhou et al. [85]	China	×	–	50	50	U[b]	U[b]	U[b]
Zhou et al. [86]	China	×	–	71	61	61	0	0
Zhou et al. [87]	China	×	–	70	70	70	0	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Pancreatic cancer only								
Akita et al. [88]	Japan	×	–	116	138	138	0	0
Balasenthil et al. [89]	USA	–	×	98	154	61	93	0
Brand et al. [90]	USA	×	–	173	120	120	0	0
Cao et al. [91]	China	×	–	156	115	0	57	58
Capello et al. [92]	USA	×	–	73	134	60	74	0
Chung et al. [93]	South Korea	×	–	55	93	70	23	0
Chung et al. [94]	South Korea	×	–	54	80	55	25	0
Deng et al. [95]	China	×	–	303	640	600	40	0
Duraker et al. [96]	Turkey	×	–	123	173	0	173	0
Firpo et al. [97]	USA	×	×	75	261	150	84	27
Fukutake et al. [98]	Japan	×	–	240	7800	7772	28	0
Gao et al. [99]	China	×	–	70	120	50	70	0
Gold et al. [100]	USA	–	×	53	130	43	87	0
Gold et al. [101]	USA	×	×	298	199	79	120	0
Groblewska et al. [102]	Poland	U	U	62	65	65	0	0
Guo et al. [103]	China	×	–	250	300	150	150	0
Honda et al. [104]	Japan, Germany	×	–	319	291	181	110	0
Honda et al. [105]	Japan, USA	×	×	384	342	192	150	0
Honda et al. [106]	Ten European countries[c]	–	×	156	213	213	0	0
Jiang et al. [107]	China	×	–	96	252	200	52	0
Kaur et al. [108]	USA	×	–	154	167	0	167	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Kim et al. [109]	USA	×	×	278	418	220	83	115
Kuwatani et al. [110]	Japan	×	–	98	158	105	21	32
LeCalvez-Kelm et al. [111]	Czech Republic, Slovakia	×	×	397	533	374	159	0
Lee et al. [112]	South Korea	×	–	51	112	0	112	0
Liao et al. [113]	Taiwan	×	×	58	146	102	44	0
Liu et al. [114]	China	×	–	138	175	68	107	0
Liu et al. [115]	China	×	–	172	215	133	82	0
Liu et al. [116]	China	–	×	235	470	240	230	0
Matsubara et al. [117]	Japan	×	–	140	97	87	0	10
Mayerle et al. [118]	Germany	–	×	79	160	80	80	0
Mellby et al. [119]	Denmark, USA	–	×	143	276	219	57	0
Mizuno et al. [120]	Japan	×	–	180	180	84	96	0
O'Brien et al. [121]	UK	–	×	101	184	184	0	0
Park et al. [122]	South Korea	–	×	139	146	74	72	0
Park et al. [123]	South Korea	U	U	292	165	94	71	0
Peng et al. [124]	Taiwan	×	×	263	230	185	45	0
Poruk et al. [125]	USA	×	×	86	134	86	48	0
Ritchie et al. [126]	Canada	–	×	84	99	99	0	0
Rychlikova et al. [127]	Czech Republic	×	–	64	185	48	137	0
Sakai et al. [128]	Japan	×	–	53	147	102	22	23
Song et al. [129]	USA	–	×	188	220	89	68	63
Tachezy et al. [130]	Germany	×	×	116	243	128	115	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Talar-Wojnarowska et al. [131]	Poland	×	–	85	122	50	72	0
Tavano et al. [132]	Italy	×	–	74	117	117	0	0
Ward et al. [133]	UK	×	–	75	61	0	61	0
Xu et al. [134]	China	×	–	156	180	65	57	58
Zhang et al. [135]	China	×	–	129	278	183	95	0
Zhang et al. [136]	China	×	–	67	206	145	61	0
Zhong et al. [137]	China	×	–	183	202	141	61	0
Zhou et al. [138]	China	×	–	152	207	96	91	20
Zhou et al. [139]	China	×	–	156	199	163	36	0
Zhou et al. [140]	China	×	–	64	64	64	0	0
Oesophageal cancer only								
Bagaria et al. [141]	India	×	–	50	50	50	0	0
Bai et al. [142]	China	×	–	89	125	80	14	31
Bagaria et al. [143]	India	×	–	50	50	50	0	0
Brockmann et al. [144]	Germany	×	–	50	150	50	100	0
Huang et al. [145]	China	×	–	60	60	60	0	0
Jia et al. [146]	China	×	–	101	98	98	0	0
Liao et al. [147]	China	×	–	151	230	194	36	0
Lukaszewicz-Zajac et al. [148]	Poland	×	–	56	65	65	0	0
Lv et al. [149]	China	×	–	126	80	80	0	0
Pan et al. [150]	China	×	–	50	110	60	50	0
Peng et al. [151]	China	×	–	104	53	53	0	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Sudo et al. [152]	Japan	×	×	283	9364	9203	161	0
Wang et al. [153]	China	×	–	84	154	154	0	0
Xing et al. [154]	China	×	–	169	154	80	74	0
Xu et al. [155]	China	×	–	237	134	134	0	0
Xu et al. [156]	China	×	–	70	80	80	0	0
Yan et al. [157]	China	×	–	364	229	229	0	0
Zhang et al. [158]	China	×	–	81	81	81	0	0
Zhang et al. [159]	China	×	–	62	58	58	0	0
Zhang et al. [160]	China	×	–	81	81	81	0	0
Zhang et al. [161]	China	×	–	186	186	186	0	0
Zhang et al. [162]	China	×	–	112	112	112	0	0
Zheng et al. [163]	China	×	–	150	185	126	59	0
Zhou et al. [164]	China	–	×	88	479	200	0	279
Biliary tract cancers only								
Deng et al. [165]	China	×	–	153	65	0	65	0
Leelawat et al. [166]	Thailand	×	–	59	128	0	128	0
Wang et al. [167]	China	×	–	78	156	78	78	0
More than one tumour type								
Bagaria et al. [168]	India	×	–	50 GC 50 OC	50	50	0	0
Markar et al. [169]	UK	×	–	163 GC or OC	172[d]	89	82	0

Table 1 continued

References	Country (population)	Setting[a]		Cases and controls				
				Cases (N)	Controls (N)			
		Hosp	Other		All	HC	NM	PM
Ren et al. [170]	China	×	–	1049 GC 268 OC 160 PaC	1019	747	272	0
Schneider et al. [171]	Germany	U	U	122 GC 86 OC	53	53	0	0

GC gastric cancer, *HC* healthy control, *Hosp* hospital, *NM* non-malignant, *OC* oesophageal cancer, *PaC* pancreatic cancer, *PM* pre-malignant, *U* unclear, *UK* United Kingdom, *USA* United States of America

[a] Due to wide variations in health systems across different countries, hospital setting is a broad definition than can encompass secondary and tertiary care. Other setting refers to biobanks, reference sets, databases, or archived samples; general population cohorts or cohorts from population screening programmes; or cohorts from previous trials or observational studies

[b] In most of these studies, unclear numbers refer to healthy controls and non-malignant conditions combined (70 controls for [26], 120 controls for [42], 60 controls for [49], and 70 controls for [50]). In the case of Zhou et al. [85], it is also unclear whether controls had pre-malignant conditions

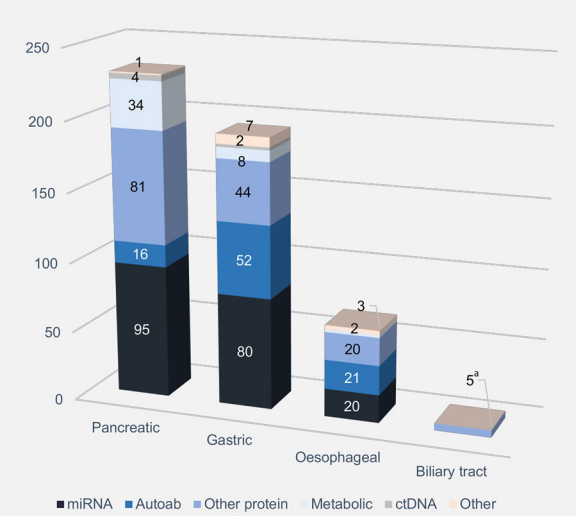
[c] Denmark, France, Italy, Germany, Greece, Spain, UK, Norway, Sweden & Netherlands

[d] Sum of controls does not add up to total number of controls (mismatch in paper)

distinguishing between oesophageal and gastric cancer). The minimum age for cases was 16 while the oldest patient was aged 93. Most cases were male (68%) across all tumour types. Over 50% of cancers had been diagnosed at stages III and IV (median 55.5%, interquartile range 47.0–68.1%; data available for 106 included studies). The included studies reported on 49,474 controls (38,955 normal/healthy, 9042 with non-malignant conditions, 1106 with pre-malignant conditions, and 371 with either normal or non-malignant conditions). Pancreatitis and gastritis were the most commonly reported non-malignant conditions (online supplementary Figure S1). Over half of the studies ($n = 83$) investigated more than one type of control population. Normal healthy controls were the majority across all tumour types, except for biliary tract cancers. The minimum age for controls was 16 while the maximum age was 94. Overall, most controls were male (74%); this was the case for all tumour types except for biliary tract cancers.

Types of Biomarkers

Biomarkers were most commonly sampled from blood (145 studies; 107 investigated serum, 33 plasma and 5 both); two studies analysed urine [28, 36], one breath [169] and another saliva [47]. Most studies ($n = 128$) investigated more than one biomarker. A total of 431 biomarkers were identified (online supplementary table S2). These were most often microRNA and other RNAs ($n = 183$), other proteins ($n = 119$) and autoantibodies and other immunological markers ($n = 79$). Less than a third of studies ($n = 44$) included biomarkers from different categories. This was often due to use of established biomarkers (proteins CA19-9 and CEA) in combination with novel biomarkers. Studies of pancreatic cancer reported on over half of identified biomarkers ($n = 231$) (Fig. 3). Only about a fifth ($n = 90$) of all identified biomarkers were reported in more than one study; 72 of these were reported in more than one study for the same tumour type (Table 3).



Identified biomarkers (149 studies)	N (%)
MicroRNAs and other RNAs (including protein coding genes)	183 (42.5%)
Autoantibodies and other immunological markers	79 (18.3%)
Other proteins	119 (27.6%)
Adhesion and Matrix Proteins	15 (12.6%)
Classic Tumour Markers	13 (10.9%)
Coagulation and angiogenesis molecules	7 (5.9%)
Cytokines, Chemokines and Insulin-like Growth Factors	23 (19.3%)
Hormones	3 (2.5%)
Novel Proteins	29 (24.4%)
Not otherwise specified	29 (4.4%)
Metabolic markers	36 (8.4%)
Circulating tumour DNA	6 (1.4%)
Other biomarkers[b]	8 (1.9%)

Fig. 3 Types of biomarkers, overall and by tumour type. [a]Five proteins; [b]these refer to volatile organic compounds and platelets; *autoab* autoantibodies, *ctDNA* circulating tumour DNA, *miRNA* microRNA

Measures of Diagnostic Performance

The most commonly reported measures of diagnostic performance were sensitivity ($n = 136$), specificity ($n = 129$) and AUC ($n = 123$). PPV and NPV were each reported by 40 studies, while false positives and false negatives were least often reported (11 studies each). Outcome data on individual biomarkers were available in most studies ($n = 121$); the remaining 28 studies only reported on performance for a combination/panel. Over half of the included studies ($n = 83$) reported on measures of performance for biomarkers both individually and in combinations. Outcome data were not available for all control populations; only 95 studies provided outcome data for cancers versus normal controls, 54 provided outcome data for cancers versus non-malignant controls, and 10 provided measures for cancers

Table 2 Characteristics of included studies: biomarkers and study design

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Gastric cancers only														
Cai et al. [23]	15	–	–	–	–	–	–	×	–	×	–	–	×	–
Chen et al. [24]	–	–	1	–	–	–	×	–	–	×	–	U	U	U
Chen et al. [25]	–	–	4	–	–	–	×	–	–	×	×	–	×	×
Chung et al. [26]	–	–	2	–	–	–	×	–	–	×	×	U	×	U
Ding et al. [27]	4	–	1	–	–	–	×	–	–	×	×	–	×	–
Dong et al. [28]	–	–	1	–	–	–	–	–	×	×	–	–	×	–
Gantuya et al. [29]	–	–	2	–	–	–	×	–	–	×	×	×	–	–
Gwak et al. [30]	–	–	5	–	–	–	×	–	–	×	–	–	–	×
He et al. [31]	–	–	4	–	–	–	×	–	–	×	×	U	×	U
Hoshino et al. [32]	–	6	2	–	–	–	×	–	–	×	×	–	×	–
Huang et al. [33]	–	1	5	–	–	–	×	–	–	×	–	–	×	×
Huang et al. [34]	5	–	2	–	–	–	×	–	–	×	×	–	×	–
Huang et al. [35]	5	–	–	–	–	–	×	–	–	–	×	U	U	U
Iwasaki et al. [36]	2	–	–	–	–	–	–	–	×	×	–	–	×	–
Ji et al. [37]	2	–	–	–	–	–	–	×	–	×	–	–	MB	–
Juan Cai et al. [38]	–	–	3	–	–	–	×	–	–	×	–	–	MB	MB
Kaise et al. [39]	–	1	5	–	–	–	×	–	–	×	×	–	×	–
Kang et al. [40]	–	–	1	–	–	–	×	–	–	×	–	×	–	–
Kikuchi et al. [41]	–	–	2	–	–	–	×	–	–	×	×	×	–	–
Kim et al. [42]	1	–	–	–	–	–	×	–	–	×	–	–	×	×
Kurilovich et al. [43]	–	1	2	–	–	–	×	–	–	×	×	–	×	–

Table 2 continued

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Li et al. [44]	3	–	–	–	–	–	–	×	–	×	×	U	U	U
Li et al. [45]	1	–	–	–	–	–	–	×	–	×	–	U	×	U
Li et al. [46]	3	–	4	–	–	–	–	×	–	×	–	–	×	–
Li et al. [47]	13	–	–	–	–	–	–	–	×	×	×	–	×	–
Li et al. [48]	–	–	5	–	–	–	×	–	–	×	×	MB	–	–
Lim et al. [49]	–	–	3	–	–	–	×	–	–	×	×	U	×	×
Lim et al. [50]	–	–	3	–	–	–	×	–	–	×	×	MB	×	×
Lin et al. [51]	2	–	–	–	–	–	×	×	–	×	–	U	MB	U
Liu et al. [52]	2	–	2	–	–	–	×	–	–	–	×	–	×	–
Liu et al. [53]	–	–	4	–	–	–	×	–	–	×	×	–	×	×
Liu et al. [54]	3	–	–	–	–	–	–	×	–	–	×	–	×	–
Meistere et al. [55]	–	18	–	–	–	–	×	–	–	–	×	–	×	–
Mroczko et al. [56]	–	–	3	–	–	–	×	×	–	×	–	–	×	–
Ning et al. [57]	–	–	4	–	–	–	×	–	–	×	×	–	×	–
Oue et al. [58]	–	–	4	–	–	–	×	–	–	×	×	–	×	×
Pan et al. [59]	–	1	5	–	–	–	×	×	–	×	×	U	×	U
Park et al. [60]	–	–	–	–	2	–	–	×	–	×	×	–	×	×
Parvaee et al. [61]	3	–	–	–	–	–	–	×	–	×	–	–	×	–
Qin et al. [62]	–	9	–	–	–	–	×	–	–	×	×	–	×	–
Qiu et al. [63]	4	–	–	–	–	–	–	×	–	×	×	U	U	U
Song et al. [64]	8	–	–	–	–	–	×	–	–	×	×	×	–	–
Su et al. [65]	–	–	5	–	–	–	×	–	–	–	×	–	×	×

Table 2 continued

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Sun et al. [66]	–	1	3	–	–	–	×	–	–	×	×	MB	–	–
Tsalikidis et al. [67]	–	–	1	–	–	–	×	–	–	×	–	–	×	–
Wang et al. [68]	–	–	1	–	–	–	×	–	–	×	–	–	×	–
Wang et al. [69]	5	–	–	–	–	–	×	–	–	–	×	U	U	U
Wang et al. [70]	–	6	–	–	–	–	×	–	–	–	×	–	×	–
Wang et al. [71]	–	–	3	–	–	–	×	–	–	×	×	U	×	U
Werner et al. [72]	–	14	–	–	–	–	×	–	–	–	×	–	×	–
Wu et al. [73]	1	–	2	–	–	–	×	–	–	×	–	U	U	U
Wu et al. [74]	–	–	4	–	U	–	×	–	–	×	–	–	×	×
Wu et al. [75]	–	–	1	–	–	3	×	–	–	×	×	U	×	U
Yanaoka et al. [76]	–	–	2	–	–	–	×	–	–	×	×	×	–	–
Yang et al. [77]	–	–	1	–	–	–	–	×	–	×	–	×	–	–
Yang et al. [78]	3	–	5	–	–	–	–	×	–	×	×	–	–	×
Yoon et al. [79]	–	–	1	–	–	–	×	–	–	×	–	–	×	–
Yun et al. [80]	–	–	1	–	–	2	×	–	–	×	×	MB	×	MB
Zayakin et al. [81]	–	45	–	–	–	–	×	–	–	–	×	–	×	×
Zhang et al. [82]	–	–	–	6	–	–	×	–	–	×	×	–	×	×
Zhang et al. [83]	1	–	–	–	–	–	–	×	–	×	–	–	–	×
Zhang et al. [84]	5	–	4	–	–	–	–	×	–	×	×	–	–	×
Zhou et al. [85]	1	–	–	–	–	–	–	×	–	×	–	U	U	U
Zhou et al. [86]	5	–	–	–	–	–	–	×	–	–	×	U	U	U
Zhou et al. [87]	1	–	–	–	–	–	–	×	–	×	–	U	U	U

Table 2 continued

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Pancreatic cancers only														
Akita et al. [88]	–	–	–	4	–	–	×	–	–	×	×	U	U	U
Balasenthil et al. [89]	–	–	3	–	–	–	–	×	–	–	×	×	–	–
Brand et al. [90]	–	–	3	–	–	–	×	–	–	×	×	–	×	×
Cao et al. [91]	6	–	–	–	–	–	–	×	–	–	×	U	U	U
Capello et al. [92]	6	–	2	–	–	–	–	×	–	×	×	U	U	U
Chung et al. [93]	–	2	–	1	–	–	×	–	–	×	×	U	×	U
Chung et al. [94]	–	1	20	–	–	1	×	–	–	×	×	×	×	–
Deng et al. [95]	1	–	–	–	–	–	×	–	–	×	–	U	U	U
Duraker et al. [96]	–	–	3	–	–	–	×	–	–	×	×	U	U	U
Firpo et al. [97]	–	–	3	–	–	–	×	–	–	×	×	MB	×	MB
Fukutake et al. [98]	–	–	–	6	–	–	–	×	–	–	×	–	×	×
Gao et al. [99]	1	–	1	–	–	–	×	–	–	×	×	U	×	U
Gold et al. [100]	–	–	1	–	–	–	×	–	–	×	–	–	×	×
Gold et al. [101]	–	1	1	–	–	–	×	–	–	×	×	U	×	U
Groblewska et al. [102]	–	–	4	–	–	–	×	–	–	×	×	–	×	–
Guo et al. [103]	–	–	2	–	–	–	×	–	–	×	×	U	×	U
Honda et al. [104]	–	–	4	–	–	–	–	×	–	×	×	×	–	–
Honda et al. [105]	–	–	3	1	–	–	–	×	–	×	×	×	–	–
Honda et al. [106]	–	–	3	–	–	–	–	×	–	×	×	×	–	–
Jiang et al. [107]	–	–	3	–	–	–	×	–	–	×	×	–	×	×
Kaur et al. [108]	–	1	–	–	–	–		×	–	×	–	×	–	–

Table 2 continued

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Kim et al. [109]	–	–	2	–	–	–	×	×	–	×	×	–	×	×
Kuwatani et al. [110]	–	–	3	–	–	–	×	–	–	×	×	U	U	U
LeCalvez-Kelm et al. [111]	–	–	–	–	3	–	–	×	–	–	×	U	×	U
Lee et al. [112]	–	–	6	–	–	–	×	–	–	×	×	U	U	U
Liao et al. [113]	–	–	2	–	–	–	×	–	–	×	×	–	×	×
Liu et al. [114]	7	–	1	–	–	–	–	×	–	×	×	MB	×	MB
Liu et al. [115]	7	–	–	–	–	–	×	–	–	–	×	–	×	×
Liu et al. [116]	–	–	11	–	–	–	×	–	–	×	×	–	×	×
Matsubara et al. [117]	–	–	2	–	–	–	–	×	–	×	×	U	MB	U
Mayerle et al. [118]	–	–	1	9	–	–	–	×	–	–	×	MB	–	MB
Mellby et al. [119]	1	5	20	3	–	–	×	–	–	–	×	×	–	–
Mizuno et al. [120]	–	–	–	6	–	–	–	×	–	–	×	–	×	×
O'Brien et al. [121]	1	–	3	–	–	–	×	–	–	×	×	×	–	–
Park et al. [122]	–	–	9	–	–	–	×	–	–	×	×	U	MB	U
Park et al. [123]	–	–	5	–	–	–	×	–	–	×	×	U	×	×
Peng et al. [124]	–	–	2	–	–	–	×	–	–	×	×	–	×	×
Poruk et al. [125]	–	–	3	–	–	–	×	–	–	×	×	–	×	MB
Ritchie et al. [126]	–	–	1	1	–	–	×	–	–	×	×	U	U	U
Rychlikova et al. [127]	–	–	4	–	–	–	×	–	–	×	×	MB	U	MB
Sakai et al. [128]	56	–	2	–	–	–	×	×	–	×	×	–	×	MB
Song et al. [129]	–	3	3	–	–	–	×	–	–	×	×	U	U	U
Tachezy et al. [130]	1	–	–	–	–	–	×	–	–	×	–	U	×	U

Table 2 continued

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Talar-Wojnarowska et al. [131]	–	1	1	–	–	–	×	–	–	×	–	U	MB	U
Tavano et al. [132]	1	–	1	–	–	–	×	–	–	×	×	×	–	–
Ward et al. [133]	–	–	1	2	–	–	×	–	–	×	×	U	U	U
Xu et al. [134]	8	–	–	–	–	–	–	×	–	×	–	U	U	U
Zhang et al. [135]	–	2	3	1	–	–	×	–	–	–	×	U	U	U
Zhang et al. [136]	–	–	–	6	–	–	×	–	–	×	×	U	×	U
Zhong et al. [137]	–	1	1	–	–	–	×	–	–	×	×	U	U	U
Zhou et al. [138]	–	1	2	–	–	–	×	–	–	×	×	×	–	–
Zhou et al. [139]	–	–	2	–	–	–	×	–	–	×	×	U	U	U
Zhou et al. [140]	6	–	–	–	–	–	–	×	–	–	×	–	×	–
Oesophageal cancers only														
Bagaria et al. [141]	–	–	1	–	–	–	×	–	–	×	–	U	U	U
Bai et al. [142]	1	–	1	–	–	–	–	×	–	×	×	–	×	×
Bagaria et al. [143]	–	–	4	–	–	–	×	–	–	×	–	–	×	–
Brockmann et al. [144]	–	2	2	–	–	–	×	–	–	×	–	–	×	×
Huang et al. [145]	5	–	–	–	–	–	×	–	–	–	×	–	MB	–
Jia et al. [146]	1	–	6	–	–	–	×	–	–	–	×	–	×	–
Liao et al. [147]	–	–	4	–	–	–	–	×	–	×	×	U	U	U
Lukaszewicz-Zajac et al. [148]	–	–	2	–	–	–	×	–	–	×	×	–	×	–
Lv et al. [149]	2	–	–	–	–	–	×	–	–	×	×	–	×	–
Pan et al. [150]	–	4	–	–	–	–	×	–	–	×	×	U	×	U
Peng et al. [151]	–	1	1	–	–	–	×	–	–	×	×	–	MB	–

Table 2 continued

References	Biomarkers											Design		
	Type (*N*)						Sample			Report		Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Sudo et al. [152]	6	–	–	–	–	–	×	–	–	–	×	–	×	×
Wang et al. [153]	1	–	–	–	–	–	×	–	–	×	–	U	U	U
Xing et al. [154]	2	–	1	–	–	–	×	–	–	×	×	–	×	×
Xu et al. [155]	–	5	1	–	–	–	×	–	–	–	×	–	×	–
Xu et al. [156]	–	5	1	–	–	–	×	–	–	–	×	–	×	–
Yan et al. [157]	–	–	1	–	–	–	×	–	–	×	–	–	×	–
Zhang et al. [158]	1	–	–	–	–	–	×	–	–	×	–	U	U	U
Zhang et al. [159]	–	1	–	–	–	–	×	–	–	×	–	U	U	U
Zhang et al. [160]	1	–	–	–	–	–	×	–	–	×	–	U	U	U
Zhang et al. [161]	–	6	–	–	–	–	×	–	–	–	×	–	×	–
Zhang et al. [162]	–	2	–	–	–	–	×	–	–	×	×	U	U	U
Zheng et al. [163]	–	–	4	–	–	–	×	–	–	×	×	–	×	×
Zhou et al. [164]	–	8	–	–	–	–	×	–	–	×	×	–	–	×
Biliary tract cancers only														
Deng et al. [165]	–	–	4	–	–	–	×	–	–	×	×	–	–	×
Leelawat et al. [166]	–	–	2	–	–	–	×	–	–	×	–	×	–	–
Wang et al. [167]	–	–	4	–	–	–	×	–	–	×	×	MB	×	–
More than one tumour type														
Bagaria et al. [168]	–	–	2	–	–	–	×	–	–	×	×	–	×	–
Markar et al. [169]	–	–	–	–	–	5	–	–	×	–	×	MB	–	–
Ren et al. [170]	–	1	2	–	–	–	×	–	–	×	×	U	U	U

Table 2 continued

References	Biomarkers						Sample			Report		Design		
	Type (*N*)											Sgl	2-gate	
	miRNA	Autoab	Protein	Metab	ctDNA	Other[a]	Serum	Plasma	Other[b]	Ind	Comb	RFD	TGN	TGA
Schneider et al. [171]	–	–	4	–	–	–	×	–	–	×	×	–	×	–

autoab autoantibodies and other immunological markers, *Comb* combination or panel, *ctDNA* circulating tumour DNA, *Ind* individual, *MB* maybe/likely (design likely but no sufficient information to make a final decision), *metab* metabolic markers, *RFD* reversed-flow design, *Sgl* single-gate design, *TGA* two-gate alternative diagnosis, *TGN* two-gate normal, *U* unclear

[a] Other biomarker type refers to volatile organic compounds or platelets

[b] Other sample refers to urine or volatile organic compounds

versus pre-malignant conditions (online supplementary table S3).

Individual measures of diagnostic performance were available for 35 biomarkers mentioned more than once, for the same tumour type (online supplementary table S4). We were not able to synthesise outcomes further due to heterogeneity in biomarker combinations, in control populations and subgroup analyses, and variations in reported cut-off points and diagnostic accuracy data (see online supplementary table S5 for a textual description of outcomes).

Only four novel biomarkers were reported on studies adopting a single-gate design (Table 4). Apolipoproteins AII-AT and AII-ATQ had poor sensitivity (range 4–25%) but good AUCs (range 52–94.6%) reported for pancreatic cancer in three studies (same first author for all) [104–106]. Their diagnostic accuracy increased when combined with CA19-9 (sensitivity range 7–95.4%, specificity range 96–98%, AUC range 56–78%). Pepsinogen I (PGI) and PGI/PGII ratio had a wide range of sensitivity and specificity (ranges 27–77.9% and 20.2–92%, respectively) and good AUC (range 70–76%) reported for gastric cancer across four studies [29, 40, 41, 76]. When evaluated with other novel biomarkers (including miR-1290, MIC-1, ULBP2 and CA125), one established biomarker, CA19-9, also showed some promise (sensitivity range 23.1–88%, specificity range 71.6–96.6%, AUC 92–98%) for pancreatic cancer [121, 132, 138]. There were also two studies reporting panels rather than individual biomarkers using a single-gate, reversed-flow design (Table 4) [89, 119].

DISCUSSION

Our systematic review identified 149 studies reporting on 431 different biomarkers for gastric, pancreatic, oesophageal and biliary tract cancers. Only a fifth of biomarkers were reported by more than one study, and from these only four novel biomarkers, apoAII-AT and apoAII-ATQ (pancreatic cancer) and pepsinogen I and II (gastric cancer), plus one established biomarker (CA19-9 combined with other novel biomarkers), were reported with individual

Table 3 Biomarkers investigated more than once, for the same tumour type (number of studies)

Biomarker	Pancreatic cancer	Gastric cancer	Oesophageal cancer	Biliary tract cancer
MicroRNAs and other RNAs (including protein coding genes)				
miR-21	2 [114, 115]	3 [23, 34, 44]	–	–
miR-20a	–	3 [23, 52, 86]	–	–
miR-25	2 [95, 115]	2 [46, 86]	–	–
miR-296-5p	–	2 [35, 69]	–	–
miR-210	–	2 [61, 86]	–	–
miR-1	–	2 [23, 52]	–	–
miR-106b	–	2 [23, 46]	–	–
miR-106b-3p	2 [91, 134]	–	–	–
miR-126-3p	2 [91, 134]	–	–	–
miR-1285	2 [91, 134]	–	–	–
miR-132-3p	–	2 [35, 69]	–	–
miR-16	2 [99, 114]	–	–	–
miR-214	–	2 [37, 83]	–	–
miR-221	–	2 [23, 64]	–	–
miR-223	–	2 [44, 85]	–	–
miR-26b-3p	2 [91, 134]	–	–	–
miR-27a	–	2 [23, 52]	–	–
miR-376c	–	2 [23, 64]	–	–
miR-423-5p	–	2 [23, 52]	–	–
miR-486-5p	2 [91, 134]	–	–	–
miR-744	–	2 [23, 64]	–	–
miR-938	2 [91, 134]	–	–	–
REG3A	2 [92, 121]	–	–	–

Table 3 continued

Biomarker	Pancreatic cancer	Gastric cancer	Oesophageal cancer	Biliary tract cancer
Autoantibodies and other immunological markers				
p53	–	2 [32, 62]	4 [155, 156, 161, 164]	–
C-Myc	–	2 [62, 70]	2 [161, 164]	–
p62	–	2 [62, 70]	2 [161, 164]	–
New York esophageal squamous cell carcinoma 1 (NY-ESO-1 or CTAG1A)	–	–	3 [150, 155, 156]	–
Squamous Cell Carcinoma-Antigen (SCC-Antigen)	–	–	3 [144, 147, 163]	–
Antibodies against *Helicobacter pylori* (HpAb)	–	2 [39, 66]	–	–
BMI-1	–	–	2 [155, 156]	–
Heat shock protein 70 (HSP70)	–	–	2 [155, 156]	–
Immunoglobin G galactosylation ratio (IgG- Gal-ratio)	2 [137, 170]	–	–	–
IMP1	–	–	2 [161, 164]	–
Koc	–	–	2 [161, 164]	–
MIC	2 [129, 138]	–	–	–
NPM1	–	2 [62, 70]	–	–
P16	–	2 [62, 70]	–	–
Peroxiredoxin 6 (Prx6)	–	–	2 [155, 156]	–
Other proteins				
CA19-9	35[a]	20[b]	4 [143, 168, 170, 171]	–

Table 3 continued

Biomarker	Pancreatic cancer	Gastric cancer	Oesophageal cancer	Biliary tract cancer
Carcinoembryonic antigen (CEA)	7 [96, 102, 110, 112, 116, 127, 170]	27[c]	9 [141, 143, 144, 147, 148, 163, 168, 170, 171]	2 [165, 167]
CA125	4 [96, 112, 116, 121]	6 [25, 31, 59, 73, 78, 84]	–	2 [165, 167]
CA724	–	9 [25, 30, 46, 48, 53, 57, 59, 74, 171]	2 [144, 171]	–
Pepsinogen I (PGI)	–	9 [29, 33, 38–41, 43, 66, 76]	–	–
Pepsinogen II (PGII)	–	8 [29, 33, 39–41, 43, 66, 76]	–	–
Tissue Inhibitor of Metalloproteinase 1 (TIMP-1)	4 [92, 122, 123, 125]	2 [56, 68]	–	–
Alpha-Fetoprotein (AFP)	2 [112, 116]	3 [31, 59, 78]	–	–
Osteopontin	3 [125, 127, 129]	2 [24, 66]	–	–
CYFRA21-1	–	–	4 [142, 144, 147, 163]	–
Interleukin-6 (IL-6)	3 [94, 119, 135]	–	–	–
Apolipoprotein AII-AT (apoAII-AT)	3 [104–106]	–	–	–
Apolipoprotein AII-ATQ (apoAII-ATQ)	3 [104–106]	–	–	–
CA242	2 [107, 116]	–	–	–
CEACAM-1	2 [121, 129]	–	–	–
Interleukin-4 (IL-4)	2 [94, 119]	–	–	–
Interleukin-8 (IL-8 or CXCL8)	2 [94, 135]	–	–	–
Interleukin-13 (IL-13)	2 [94, 119]	–	–	–
Insulin-like growth factor-binding protein-2 (IGFBP2)	2 [92, 123]	–	–	–

Table 3 continued

Biomarker	Pancreatic cancer	Gastric cancer	Oesophageal cancer	Biliary tract cancer
Matrix metalloproteinase-7 (MMP-7)	–	–	2 [155, 156]	–
Neuron-specific enolase (NSE)	2 [112, 116]	–	–	–
Trefoil factor 1 (TFF1)	–	2 [33, 39]	–	–
Trefoil factor 2 (TFF2)	–	2 [33, 39]	–	–
Trefoil factor 3 (TFF3)	–	2 [33, 39]	–	–
Thrombospondin 2 (THBS2)	2 [109, 124]	–	–	–
Vascular Endothelial Growth Factor (VEGF)	2 [94, 119]	–	–	–
Metabolic markers				
Histidine	3 [98, 118, 120]	–	–	–
Alanine	2 [98, 120]	–	–	–
Asparagine	2 [98, 120]	–	–	–
Isoleucine	2 [98, 120]	–	–	–
PC-594	2 [88, 126]	–	–	–
Phosphatidylcholine-C18.0-C22.6	2 [88, 118]	–	–	–
Serine	2 [98, 120]	–	–	–
Tryptophan	2 [98, 120]	–	–	–

[a] CA19-9 in pancreatic cancer:[89, 90, 92, 96, 97, 99, 101–103, 105–107, 109, 110, 112–114, 116–118, 121–127, 129, 132, 133, 135, 137–139, 170]

[b] CA19-9 in gastric cancer:[25, 27, 30–32, 34, 38, 46, 52, 53, 57–59, 65, 74, 78, 84, 168, 170, 171]

[c] CEA in gastric cancer: [25, 26, 30–32, 34, 38, 46, 48–50, 52, 53, 56–59, 65, 73–75, 78, 80, 84, 168, 170, 171]

Table 4 Biomarkers reported more than once for the same tumour type and panels adopting a single-gate (reversed-flow) design

References	Recruitment setting	Cases	Controls	Outcomes (Sensitivity, specificity, AUC where available)
1. Measures of diagnostic performance available for individual biomarkers, in studies adopting a single-gate design				
Apolipoprotein AII-AT/ATQ alone and in combination with CA19-9 (pancreatic cancer)				
Honda et al. [106]	EPIC cohort (population-based study)	156 PaC Median age 58.1 (34.9–75.7) 53% male Staging: 13 localised, 73 metastatic, 69 NA	213 HC Median age 58.0 (34.5–75.4) 53% male (matched to cases)	Measures for months prior to diagnosis (lag times): up to 6 months, > 6–18, 18, > 18–36 and > 36–40 months For ApoAII-AT/ATQ alone, 2 cut-off points Sensitivity, range 0.04–0.25 AUC, range 0.52–0.62 For ApoAII-AT/ATQ plus CA19-9, 2 cut-off points Sensitivity, range 0.07–0.57 Specificity, range 0.96–0.98 AUC, range 0.56–0.78
Honda et al. [105]	Cohort 1: National Cancer Centre Hospital	131 IDACP Mean age 68.8 (9.01) 55% male Staging: most at advanced stages	131 HC Mean age 62.5 (10.8) 52% male	Measures for ELISA and mass spectrometric analysis, also according to tumour staging For ApoAII-ATQ/AT alone, 1 cut-off point AUC, range 0.856–0.946 For ApoAII-AT/ATQ plus CA19-9, 1 cut-off point each Sensitivity, 95.4% (cohort 2) Specificity, 98.3% (cohort 2)
	Cohort 2: Seven Medical Institutions	155 IDACP Age and sex NA Staging: majority advanced stages	57 pancreatic disease other than IDACP Age and sex NA	
	Cohort 3: NCI-EDRN pancreatic reference set	98 PaC Age and sex NA Staging: all early stages	62 CP, 31 acute benign biliary obstruction, 61 HC Age and sex NA	

Table 4 continued

References	Recruitment setting	Cases	Controls	Outcomes (Sensitivity, specificity, AUC where available)
Honda et al. [104]	Cohort 1: National Cancer Hospital and Medical University Hospital	Does not meet criteria as used to calculate first measures of performance	Does not meet criteria as used to calculate first measures of performance	Measures provided according to tumour staging For ApoAII-AT/ATQ alone, 1 cut-off point AUC, 0.953 (cohort 3) For ApoAII-AT/ATQ plus CIII-0, and CA19-9, 1 cut-off point (cohort 4) Sensitivity, range 91.60–94.20% Specificity, 93.22% (same for all)
	Cohort 2: National Cancer Hospital	Does not meet criteria as there were only 41 controls	Does not meet criteria as there were only 41 controls	
	Cohort 3: Department of General Surgery	52 PaC Mean age 63.1 (9.85) 56% male Staging NA	53 HC and 58 CP HC mean age 39.1 (15.6), CP 50.3 (8.9) HC 59% male, CP 74% male	
	Cohort 4: Seven Medical Institutions	249 PDAC and 18 other malignant tumour of the pancreas PDAC mean age 64.4 (9.1), other 68.3 (9.7) PDAC 59% male, other 67% male Staging NA	128 HC, 38 benign tumour/cyst and 14 CP HC mean 46.6 (16.8), benign tumour/cyst 63.5 (11.0), CP 60.2 (10.2) HC 65% male, benign tumour/cyst 45% male, CP 86% male	
Pepsinogen (PGI and PGI/II ratio) (gastric cancer)				
Gantuya et al. [29]	National Cancer Centre Hospital	50 GC (54% w/ *H. pylori*) No information on age and sex Staging NA	752 non-cancer (302 antrum limited CG and/or atrophy and 450 corpus CG and/or atrophy (77% w/ H. pylori Mean age: 53.8 (SD 1, 27–78) 31% male	For PGI, optimal cut-off point Sensitivity, 70% Specificity, 70% AUC, 0.76 For PGI/II ratio, optimal cut-off point Sensitivity, 66% Specificity, 65% AUC, 0.70

Table 4 continued

References	Recruitment setting	Cases	Controls	Outcomes (Sensitivity, specificity, AUC where available)
Kang et al. [40]	National University Hospital	380 GC (intestinal and diffuse type) Age and sex not available for cases only No information on staging	172 BGU, 119 DU, 107 dysplasia Age and sex not available for controls only	Measures according to tumour type only (intestinal or diffuse) For PGI, 1 cut-off point Sensitivity, 77.7% (intestinal), 64.7% (diffuse) Specificity, 20.2% (intestinal), 20.2% (diffuse) For PGI/II ratio, 1 cut-off point Sensitivity, 62.3% (intestinal), 55.8% (diffuse) Specificity, 61.0% (intestinal), 61.0% (diffuse)
Kikuchi et al. [41]	University Outpatient Clinic	122 GC Age: 68.2 years (9.7) 74% male Staging NA	16 GU or DU, 17 superficial gastritis, 66 CAG, 79 no abnormality Age: 56.2 years (14.9) 55% male	Measures combining normal and non-malignant conditions Negative or positive PG test For PGI and PGI/II ratio, strict or conventional cut-off point Sensitivity, 41.3% (strict), 77.9% (conventional) Specificity, 90.4% (strict), 61.8% (conventional)
Yanaoka et al. [76]	Employees in annual health screening programme	63 GC Age: 50.3–51.8 (mean range) 100% male 86% early, 14% late stages	5146 HC Mean age: 49.2 (4.7) 100% male	or PGI and PGI/II ratio, 3 cut-off points Sensitivity, range 27.0–58.7% Specificity, range 73.4–92.0%

Table 4 continued

References	Recruitment setting	Cases	Controls	Outcomes (Sensitivity, specificity, AUC where available)
2. Measures of diagnostic performance available for established biomarkers combined with novel biomarkers not shown above, in studies adopting a single-gate design				
CA19-9 (pancreatic cancer)				
O'Brien et al. [121]	UKCTOCS screening cohort	101 PaC Age NA for validation 100% female Staging NA	184 HC Age N/A for validation 100% female	Measures according to time to diagnosis: 0–4 years, 0–2 years; 1–4 years For CA19-9 (4 cut-off points) plus CA125 (3 cut-off points) Sensitivity, range 23.1–53.1% Specificity, range 71.6–92.6%
Tavano et al. [132]	Hospital (Gastroenterology, Surgery & Oncology)	74 PaC Median age 69 (61–76) 54% male Staging NA for validation	117 HC Median age 62 (55–70) 45% male	For CA19-9 plus miR-1290, 1 cut-off point (each) Sensitivity, 83.8% Specificity, 96.6% AUC, 0.923
Zhou et al. [138]	Gastroenterology Department in Hospital	152 PaC Mean age 56 (SD 13.5) 67% male Staging: 5 IA, 12 IB, 36 IIA, 20 IIB, 40 III, 39 IV	96 HC, 91 CP, 20 pre-malignancies Mean age: HC 58 (7.6), CP 58 (15.0), pre-malignancies 60 (11.3) HC 75% male; CP 57% male; pre-malignancy 75% male	For CA19-9 plus MIC-1 and ULBP2, 1 cut-off point (each) AUC 0.982 (PaC and CP only) For CA19-9 plus MIC-1, 1 cut-off point (each) AUC 0.932 (PaC and CP only) For CA19-9 plus ULBP2, 1 cut-off point (each) AUC 0.953 (PaC and CP only)

Table 4 continued

References	Recruitment setting	Cases	Controls	Outcomes (Sensitivity, specificity, AUC where available)
3. Measures of diagnostic performance available for a panel only in studies adopting a single-gate design (all reversed-flow)				
Different panels (pancreatic cancer)[a]				
Balasenthil et al. [89]	NCI-EDRN pancreatic reference set	98 PaC (52 w/o diabetes or pancreatitis) Age and sex not available Staging: 7 IA, 8 IB, 1 II, 40 IIA and 42 IIB	62 CP, 31 acute biliary obstruction, 61 HC (50 w/o diabetes or pancreatitis) Age and sex not available	Measures for PaC vs. HC, PaC vs. CP, PaC w/o diabetes or pancreatitis vs. HC w/o diabetes or pancreatitis, and according to staging For CA19-9 plus TFPI and TNC-FN III-C, 2 cut-off points Sensitivity, range 0.73–0.81 Specificity, range 0.71–0.84 AUC, range 0.75–0.89
Mellby et al. [119]	Patients referred to Medical Centre for symptomatic pancreatic disease	2 cohorts; one for validation (US cohort) 143 PaC patients Median age only by staging; range 24–87 57% male Staging: 15 I, 75 II, 15 III and 38 IV	219 HC, 57 CP HC median age 63.0 (24–86), CP 55.5 (32–81) HC 53% male, CP 46% male	Measures available for stages I + II combined For 29-panel signature (no established biomarkers): Sensitivity, 95% Specificity, 93% AUC, 0.963 (PaC vs. HC) and 0.840 (Pac vs. CP)

ACG atrophic chronic gastritis, *ApoAII-AT/ATQ* apolipoprotein AII-AT/ATQ, *apoCIII-0* apolipoprotein CIII-0, *BGU* benign gastric ulcer, *DU* duodenal ulcer, *CG* chronic gastritis, *CP* chronic pancreatitis, *EPIC* European Prospective Investigation into Cancer and Nutrition, *GC* gastric cancer, *GU* gastric ulcer, *IDACP* invasive ductal adenocarcinoma of pancreas, *MIC* macrophage-inhibitory cytokine 1, *MPV* mean platelet volume, *NA* not available, *NCI-EDRN* National Cancer Institute Early Detection Research Network, *PaC* pancreatic cancer, *PDAC* pancreatic ductal adenocarcinoma, *PDW* platelet distribution width, *PGI/II* serum pepsinogen I/II, *PPV* positive predictive value, *TFPI* plasma tissue factor pathway inhibitor, *NTC-FN III-C* tenascin-C, *UKCTOCS* UK Collaborative Trial of Ovarian Cancer Screening, *ULBP2* UL16 binding protein 2

[a] Leelawat et al. [166] also adopted a reversed-flow design but was not added as it was the only study investigating CA19-9 for cholangiocarcinoma

measures of diagnostic performance, adopting a recommended single-gate design. Heterogeneity in methods, populations, biomarkers, outcomes and comparisons precluded meta-analysis. Applying novel biomarkers for the early detection of upper GI cancers is therefore at an early stage of matureness: few have been extensively evaluated and evaluations have almost exclusively focussed on high-prevalence populations. Further evaluation of the most promising biomarkers in low-prevalence populations is needed before extensive adoption into routine clinical practice can be recommended.

While other reviews have investigated biomarkers used for early cancer detection [19, 172], few have considered the evidence in the context of future application of tests in low-prevalence populations, the likely target for clinical application [12, 13]. To our knowledge, this is the first review to do so for upper GI cancers. The four novel and one established biomarkers we highlight in this review were evaluated in a mix of high- and low-prevalence populations, including hospital patients, general population cohorts, screening populations (both high and average cancer risk), and patients presenting with symptoms. We did not identify any studies reporting outcomes relevant to feasibility, acceptability, benefits and harms, nor health economics as initially planned in the review protocol (i.e. phase 3 studies and beyond in the CanTest framework). The best performing biomarkers for pancreatic cancer, with an AUC between 56% and 94%, were ApoAII-ATQ/AT alone, CA19-9 plus miR-1290, MIC-1 and ULPB2, and Mellby et al.'s [119] 29-panel signature. These may be ready for trials and other phase 3 studies, single or in combination, in low-prevalence populations. We did not identify any novel biomarkers with similar AUCs for gastric, biliary tract or oesophageal cancers.

A previous review investigating the role of pepsinogens in early detection of gastric cancers reported that they had only moderate capacity to detect gastric cancer [173]. Another review on early pancreatic cancer detection highlighted that no single biomarker has yet translated to clinical use and suggested the use of 'robust panels of biomarkers' [9]. This review confirms that more research is required before we have sufficient evidence about biomarkers for upper GI cancers to warrant their adoption into clinical practice.

We identified several important methodological limitations within the biomarker studies to date. These include large numbers of biomarkers analysed in parallel during discovery studies, increasing risk of falsely positive results; limited sample sizes; evaluation of "extreme" cases; limited external, independent validation; and selective reporting for validation (several alternatives analyses and combinations, use of several cut-off points and over-optimistic interpretation of the data) [12]. Together with use of two-gate rather than recommended single-gate designs, these could all lead to over-inflated measures of performance. Population characteristics were often provided as supplementary data, with little discussion of potential selection bias and other sources of uncertainty. We also excluded relevant studies when we could not obtain sufficient information on an individual tumour type; this was the case for the CancerSeek tool [174]. Adoption of reporting guidelines [175] and development of early cancer detection collaborations [15, 18] could be useful strategies to address these issues.

This review offers a comprehensive overview of the available evidence. It benefitted from having a multidisciplinary team of experts, a broad search strategy, independent screening, and classifications checked by senior team members. Since meta-analysis was not feasible nor appropriate, we had to use text and tables to synthesise the evidence. We did not include studies investigating biomarkers as part of risk prediction models or risk assessment tools. These studies have strong potential to be used in the community and should be investigated in a separate systematic review. Recent reviews indicate that only including studies in English has minimal impact on review conclusions [176, 177]. We believe this is also the case for this review, particularly due to the overall lack of evidence on biomarkers ready to be evaluated in low-prevalence settings. Although we did not formally appraise risk of bias, we identified several quality and methodological issues, indicating that challenges already highlighted

in the literature persisted over time [12]. Finally, due to the large amount of evidence on biomarker development and evaluation, we believe the field could benefit from a "living systematic review"; this refers to high quality, up-to-date online summaries of evidence which can be constantly updated as new research becomes available [178].

The studies we identified focused on measures of diagnostic performance, which is reasonable given the phase of development for most of them. The CanTest Framework [15] can help guide studies aiming to build much needed evidence on later phases of biomarker development, focussing on impact on clinical decision-making, patient, health system and economic outcomes.

CONCLUSION

There is a large body of evidence on biomarkers being developed for the detection of upper GI cancers, but relatively few have yet to demonstrate their validity or clinical utility in settings where cancer prevalence is low. Early detection of colorectal cancer already benefits from biomarkers that can be used across different populations. This is the case for the faecal immunochemical test (FIT), which is recommended for use in primary care in Spain, Australia and the United Kingdom, in addition to being effective at mass population screening programmes, using different cut-off points [179, 180]. It took several decades from FIT development to generate evidence for its cost-effectiveness as a screening test for colorectal cancer. Its role in the assessment of patients in primary care with lower GI symptoms is still being evaluated. Biomarkers for upper GI cancer remain in their infancy but there are a few which show promise and require further evaluations. Ultimately, they may be able to contribute to improving outcomes for upper GI cancers through earlier detection.

ACKNOWLEDGEMENTS

Funding. This study and the journal's rapid service fee were supported by the CanTest Collaborative (funded by Cancer Research UK C8640/A23385) of which Fiona M. Walter is Director, Jon Emery is an Associate Director, Mike Messenger is co-investigator, and Natalia Calanzani and Garth Funston are researchers. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Paige Druce, Kristi Milley and Jon Emery are supported by the Cancer Australia Primary Care Collaborative Cancer Clinical Trials Group (PC4). Mike Messenger is funded by the NIHR Leeds In Vitro Diagnostic Co-operative (UK). No Open Access Fee was received by the journal for the publication of this article.

Other Assistance. We thank Veronica Phillips, Assistant Librarian, University of Cambridge Medical Library, and Jim Berryman, Liaison Librarian, Brownless Biomedical Library University of Melbourne for expert input when developing the search strategy.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures. Mike Messenger is a paid consultant for Cepheid Inc in the field of bladder cancer diagnostics. Garth Funston is on the Editorial Board of *Advances in Therapy* but has nothing further to disclose. Natalia Calanzani, Paige E. Druce, Claudia Snudden, Kristi M. Milley, Rachel Boscott, Dawnya Behiyat, Smiji Saji, Javiera Martinez-Gutierrez, Jasmeen Oberoi, Jon Emery and Fiona M Walter have nothing to disclose.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any studies with human

participants or animals performed by any of the authors.

Data Availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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